REMARKS

1. Preliminary Remarks

a. Status of the Claims

Claims 23, 25, 31, and 33 are pending and under active consideration in this application.

b. Information Disclosure Statement

On page 2 of the Office Action, the Examiner objects to the information disclosure statement filed on March 17, 2008 because it lacked the titles of the listed publications, which were designated C1 through C10. The information disclosure statement submitted herewith includes the publication titles. Applicant notes that copies of the references C1 through C10 were already submitted on March 17, 2008.

2. Patentability Remarks

a. 35 U.S.C. §§ 101 and 112, first paragraph

On pages 3-13, the Examiner rejects claims 23, 25, 31, and 33 under 35 U.S.C. § 101 for allegedly lacking a credible asserted utility. On the basis of this rejection, the Examiner also rejects these claims under 35 U.S.C. § 112, first paragraph. The rejection under 35 U.S.C. § 101 is based on the Examiner's contention that there are no biologically relevant data readily found in the specification to show that the claimed nucleic acids are actually produced in any cell or organism, or even if produced artificially, would lead to any biological effect of any immediate, real world value.[1] The Examiner suggests that the claimed nucleic acids would meet the requirements for utility if they actually inhibit a known gene with a known function, and actually inhibit the expression of any gene.[2]

Applicant herewith presents data showing that nucleic acids related to a miRNA–hsa-miR-497–are expressed in cells and are capable of reducing expression levels of the target gene uracil DNA glycosylase ("UNG").[3] Applicant submits that this evidence establishes that the claimed subject matter has specific, substantial and credible utility. In order to satisfy the utility

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[1] *See* Instant Office Action, at 4.

[2] *See Id.*

[3] This gene is also referred to as UDG.

requirement, a specific and substantial utility must either (i) be cited in the specification or (ii) be recognized as well as established in the art, and the utility must be credible.[4]

(1) Specific Utility

A specific utility is a utility that is specific to the particular claimed subject matter, which is in contrast to a general utility that would be applicable to a broad class of inventions.[5] Applicant respectfully submits that the application provides a specific utility for the nucleic acids related to the miRNA hsa-miR-497 (SEQ ID NO: 348) in accordance with *Fisher* and Guidelines.

In *Fisher*, the claims at issue were directed to five (5) out of more than 32,000 EST that were disclosed in the application. Each of disclosed ESTs were from a cDNA library of pooled leaf tissue isolated from a maize plant. The *Fisher* application did not disclose the location of the ESTs in the genome or the function of the underlying genes. *Fisher* asserted that the utilities for claimed ESTs were (1) serving as a molecular marker; (2) measuring the level of mRNA in a tissue sample; (3) providing a source of primers for PCR of specific genes; (4) identifying the presence or absence of a polymorphism; (5) isolating promoters via chromosome walking; (6) controlling protein expression; and (7) locating genetic molecules of other plants and organisms.[6] It is important to note that each of the utilities asserted were not limited to any specific gene, genetic location or protein.

The *Fisher* court concluded that the asserted utilities were clearly not "specific." The court explained that any EST transcribed from any gene in maize could perform the seven uses such as being a molecular marker, a primer, or measure the level of RNA in a tissue sample. In other words, nothing about the seven alleged uses separated the claimed ESTs from the vast number of other ESTs also disclosed in the application. The keystone to the lack of specific utility in *Fisher* is that the claimed ESTs **did not correlate to an underlying gene of known function found in the maize genome**.

Similar to *Fisher*, the current application discloses a large number of nucleic acid sequences. In stark contrast to *Fisher*, however, the instant application provides that each of the disclosed nucleic acids may be used to target and modulate expression of **specific** gene transcripts. Specifically, Table 7, lines 313,569-313,572, as shown below, discloses that hsa-miR-497 (GAM353678) specifically targets mRNA transcripts of the *Haemophilus influenzae* target gene UNG.

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[4] *See In re Fisher*, 421 F.3d 1365, 1371 (Fed. Cir. 2005) and the Revised Interim Utility Guideline Training Materials ("Guidelines").

[5] *See Fisher*, 421 F.3d at 1371 *and* Guidelines.

[6] *See Fisher*, 421 F.3d at 1367-1368.

GAM NAME	GAM ORGANISM	GAM RNA SEQUENCE	TARGET BS-SEQ	TARGET	TARGET REF-ID	TARGET ORGANISM	UTR	BINDING SITE DRAW (UPPER:TARGET;LOWER:GAM)	GAM POS
GAM353678	Human	CAGCAGCA CACTGTGG TTTGTA	CATATCTG CTGCTG	ung	NC_000907 f rom 186 76 to 19335 (+)	Haemophilus influenzae R d	3	-- T ------ CA ATC TGCTGCTG GT TGG ACGACGAC AT T TGTCAC	A

Consequently, the claimed nucleic acids are of a **specific and unique nature** because these nucleic acids regulate the translation of mRNAs from the **specific target gene *H.influenzae* UNG**. Accordingly, the asserted utility of the claimed invention is not vague or meaningless, and there is a well-defined public benefit to regulating *H.influenzae* UNG.

(2) Substantial Utility

To satisfy the substantial utility requirement, it must be shown that the asserted use of the claimed invention has a significant and presently available benefit to the public.[7] Applicant respectfully submits that the application provides a substantial utility for the nucleic acids related to hsa-miR-497 in accordance with *Fisher* and Guidelines.

In *Fisher*, it was admitted that the underlying genes for the ESTs had no known function. *Fisher* argued that this was irrelevant because the seven asserted uses (discussed above) were not related to the function of the underlying genes. Importantly, *Fisher* failed to provide any evidence that any of the claimed ESTs could be used for any of the asserted uses. Consequently, the *Fisher* court concluded that the claimed ESTs were "mere 'objects of use-testing,' to wit, objects upon which scientific research could be performed with no assurance that anything useful will be discovered in the end."[8]

In contrast to *Fisher*, the present application discloses that hsa-miR-497 may be used to bind and regulate mRNA transcripts of *H.influenzae* UNG.[9] In addition, UNG is known to be a basic specific DNA repair enzyme.[10] This enzyme counteracts the mutagenic effect of cytosine deaminations by removing the resulting uracil and thereby initiating the uracil excision repair pathway.[11] The effects of loss of UNG activity have been tested in *E.coli ung* mutants and in

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[7] *See Id. at* 1371 *and* Guidelines.

[8] *See Fisher*, 421 F.3d at 1373, *quoting Brenner v. Manson*, 383 U.S. 519 (1966).

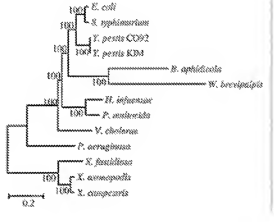
[9] *See* Instant Application, Table 7, lines 313,569-313,572.

[10] *See* Lisbeth C. Olsen, *Human uracil-DNA glycosylase complements E.coli ung mutants*, 19(16) Nucleic Acids Research 4473 (1991)("Olsen").

[11] *See Id.* at 4473 *and* Jeganathan Venkatesh, *Importance of Uracil DNA Glycosylase in* Pseudomonas aeruginosa *and* Mycobacterium smegmatis, *G+C-rich Bacteria, in Mutation Prevention, Tolerance to Acidified Nitrite, and Endurance in Mouse Macrophages*, 278(27) Jo Biol Chem 24350 (2003)("Venkatesh").

engineered *Pseudomonas aeruginosa* strains that express Ugi,[12] which is a *Bacillus subtilis* phage PBS-1-encoded UNG-inhibitor protein that is capable of inhibiting UNG in a wide variety of organisms.[13] Specifically, the effects of loss of UNG function have been tested by measuring the mutation rate in these bacteria compared to their wild-type counterparts.[14] Expression of Ugi in *P.aeruginosa* led to a 7-fold increase in mutation rate over wild-type.[15] Similarly, the *E.coli ung* mutant exhibited a 4.5-fold increase in mutation rate compared to wild-type.[16]

Applicant submits that the common effects of loss of UNG function in *E.coli* and *P.aeruginosa*, strongly suggests that inhibiting UNG function in *H.influenzae* would also result in a mutator phenotype. This is supported by the evolutionary relationship among these three bacteria, all of which belong to the gammaproteobacteria class. As shown below, *H.influenzae* is more closely related to *E.coli* than is *P.aeruginosa*.[17]



Additionally, the UNG gene and its encoded protein exhibit wide conservation among organisms, having been identified in humans, bacteria, and even viruses.[18] Equally striking, UNG protein activity is so highly conserved that the human homolog of UNG is capable of almost completely rescuing an *Escherichia coli ung* mutant.[19] Just as in bacteria, hypermutation phenotypes

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[12] *See* Venkatesh.

[13] *See* Olsen at 4473 *and* Venkatesh at 24350.

[14] *See* Venkatesh at 24353-24354.

[15] *See Id.* at 24354 (Table II).

[16] *See Id.* at 24354 (Table II).

[17] *See* Emmanuelle Lerat, *From Gene Trees to Organismal Phylogeny in Prokaryotes: The Case of the γ-Proteobacteria*, 1(1) PLoS Biology 101, 105 (2003)(Figure 5).

[18] *See* Olsen at 4473 *and* Venkatesh. For the high degree of protein sequence conservation from humans to bacteria, *see* Appendix A below.

[19] *See* Olsen.

caused by a loss of UNG function have also been observed in further-flung organisms including yeast and chickens.[20]

Accordingly, Applicant submits that in view of above evidence–the conservation of UNG across the evolutionary spectrum from viruses to humans, the highly similar effects of decreased UNG function in organisms spanning at least the spectrum from bacteria to eukaryotes, and the relatively close evolutionary relationship between *H.influenzae* and *E.coli*–inhibiting UNG function in *H.influenzae* would result in an increased mutation rate. The effects of loss of UNG function in bacteria indicates that targeting UNG is useful in controlling bacterial infections.[21] Therefore, consistent with the utility suggested by Venkatesh[22], and Applicant's asserted utility for hsa-miR-497 to target UNG mRNA as part of anti-bacterial host defense mechanism[23], Applicant submits that UNG expression could be modulated to increase *H.influenzae* mutation rate and thereby interfere with bacterial growth.

The evidence described above clearly supports that hsa-miR-497 has a number of presently available benefits to the public. One benefits is the ability to modulate the expression of UNG in order to interfere with *H.influenzae* growth. In view of the application providing a particular target of known function for hsa-miR-497, Applicant respectfully submits that the specific and substantial utility requirements are satisfied in accordance of *Fisher* and Guidelines.

(3) Credible Utility

On page 6 of the Office Action, the Examiner asserts that there would have been reason to doubt the objective truth of the asserted utility of the claimed nucleic acids because there is no experimental evidence to confirm they have any actual activity. Applicant submits herewith the declaration of Dr. Ayelet Chajut, Ph.D. under 37 C.F.R. § 1.132 (the "Chajut Declaration"), which presents experimental evidence that hsa-miR-497 is **naturally expressed**. Furthermore, data presented in the Chajut Declaration show that miRNA regulates the human homolog of the asserted target *H.influenzae* UNG.

The results of these experiments are described in the Chajut Declaration, and are summarized as follows. Dr. Chajut conducted experiments that confirmed that hsa-miR-497 is

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[20] *See* Venkatesh at 24357.

[21] *See Id.* at 24358.

[22] *See Id.* at 24358.

[23] *See* Instant Specification at Table 8, lines 687,606-687,624.

expressed in Hep3B cells.[24] Microarray experiments show that hsa-miR-497 is expressed at meaningful levels above background.[25] Thus, Applicant submits that these experiments establish that hsa-miR-497 is expressed in Hep3B cells. Additionally, confirmed expression of hsa-miR-497 as measured by microarray analysis was indicated in the specification as originally filed.[26] Applicant submits that these data show that hsa-miR-497 actually exists.

Additionally, this miRNA regulates the target mRNA produced by the UNG gene. As discussed above, the UNG gene is highly conserved across the evolutionary tree, from viruses to humans, as is the function of the encoded protein–so much so that human UNG can almost completely rescue the phenotype of a *ung* null-mutant strain of *E.coli*.[27] In addition, just like the *H.influenzae* UNG mRNA, the homologous human UNG mRNA contains a hsa-miR-497 binding site, as shown below.

hsa-mir-497::*H.influenzae* UNG target interaction

```
H.influenzae UNG            --  T   ------
                              CA ATC      TGCTGCTG
                              GT TGG      ACGACGAC
hsa-miR-497 (3'>5')         AT  T   TGTCAC
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hsa-miR-497::Human UNG target interaction

```
Human UNG 3'UTR     CCCTAGTTTGGCG  ------       C
                                CC       TGCTGCT
                                GG       ACGACGA
hsa-miR-497 (3'>5')          TGTTT  TGTCAC       C
```

As can be seen from the hsa-miR-497::UNG target interactions depicted above, both *H.influenzae* and the human UNG target mRNA interact with the seed portion of hsa-miR-497.[28] Specifically, nucleotides 1-8 of the 5' end of hsa-miR-497 bind to the target *H.influenzae* UNG mRNA (highlighted in grey). Similarly, nucleotides 2-8 of hsa-miR-497 bind to the target human UNG mRNA (also highlighted in grey). This is entirely consistent with the observation that "most known invertebrate miRNA target sites have 7 nt Watson-Crick seed matches," and that "[p]airing to the 5' portion of the miRNA, **particularly nucleotides 2-8**, appears to be most important for target

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[24] The Chajut Declaration at item 4.

[25] *Id.*

[26] *See* Instant Specification, Table 12, line 177.

[27] *See* Olsen at 4473.

[28] *See* Benjamin P. Lewis, *Prediction of Mammalian MicroRNA Targets*, 115 Cell 787, 787-788 (2003)("Lewis," of record).

recognition by vertebrate miRNAs" (emphasis added).[29] Based on the conservation of the UNG gene and the function of its encoded protein between *H.influenzae* and humans, in addition to the conservation of the hsa-miR-497 binding site between the bacterial and human UNG mRNAs, the ability of hsa-miR-497 to inhibit UNG expression was confirmed in human cells.

Dr. Chajut supervised and conducted experiments yielding results that are consistent with an ability of hsa-miR-497 to bind to and regulate the target human UNG.[30] Specifically, the experiments entailed transfecting an anti-sense oligonucleotide (ASO) specific to hsa-miR-497 into Hep3B cells that express this miRNA, and then comparing the mRNA levels of human UNG to levels in cells that were not transfected with the ASO.[31] Messenger RNA levels were measured using quantitative reverse transcription polymerase chain reactions ("qRT-PCR") and expressed as a 50-Ct cycle threshold value.[32]

The experiments are based on the following logic: if Hep3B cells do not express hsa-miR-497 and this nucleic acid does not target human UNG, then one of skill would predict that transfecting Hep3B cells with the ASO would have no effect on human UNG expression. On the other hand, if Hep3B cells do express hsa-miR-497 and this nucleic acid inhibits expression of human UNG, then one of skill would expect that transfecting a cell with the anti-hsa-miR-497 ASO would lead to an increase in the level of human UNG. The experiments described in the Chajut Declaration demonstrate that transfecting Hep3B cells with the ASO results in a 1.6-fold increase in the level of human UNG mRNA compared to cells transfected with no ASO.[33] Accordingly, these results are consistent with those one of skill would predict for a cell that expresses hsa-miR-497 and for a miRNA that targets human UNG mRNA. In view of the compelling evidence that (i) hsa-miRNA-497 decreases human UNG mRNA levels; (ii) both human UNG and *H.influenzae* UNG contain a conserved hsa-miR-497 binding site, particularly within the miRNA seed::target mRNA binding region of hsa-miR-497; and, (iii) the UNG gene and the function of its encoded protein are highly conserved across a wide evolutionary spectrum, Applicant submits that one of skill would believe that not only is hsa-miR-497 capable of inhibiting human UNG expression, but also

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[29] *Id.*

[30] *See* the Chajut Declaration at items 5-6.

[31] *See Id.* at item 5.

[32] *See Id.*

[33] *See Id.* at item 6.

H.influenzae UNG expression. In view of the foregoing, Applicant submits that nucleic acids related to hsa-miR-497 have a credible utility.

In view of the foregoing evidence of specific, substantial, and credible utility for the claimed nucleic acid and variants thereof, Applicant requests that the Examiner reconsider and withdraw the rejection of the claims under 35 U.S.C. § 101. Additionally, because hsa-miR-497 is supported by a specific, substantial, and credible utility, Applicant respectfully requests that the Examiner reconsider and withdraw the claim rejections under 35 U.S.C. § 112, first paragraph.

3. Conclusion

Applicant respectfully submits that the instant application is in good and proper order for allowance and early notification to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the instant application, the Examiner is encouraged to call the undersigned at the number listed below.

Respectfully submitted,

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Dated: November 24, 2008

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APPENDIX A

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CLUSTAL W (1.83) multiple sequence alignment


H_influenzae      ------------------------------------------------------------
E_coli            ------------------------------------------------------------
H_sapiens         MGVFCLGPWGSGRKLRTPGKGPLQLLSRLCGDHLQAIPAKKAPAGQEEPGTPPSSPLSAE


H_influenzae      -------------------------MKNWTDVIGTEKAQPYFQHTLQQVHLARASGKTIY
E_coli            ----------------------MANELTWHDVLAEEKQQPYFLNTLQTVASERQSGVTIY
H_sapiens         QLDRIQRNKAAALLRLAARNVPVGFGESWKKHLSGEFGKPYFIKLMGFVVEERK-HYTVY
                                          .* . :. *  :*** : :  *   *    *:*

H_influenzae      PPQEDVFNAFKYTAFEDVKVVILGQDPYHGPNQAHGLAFSVKPEVAIPPSLLNIYKELTQ
E_coli            PPQKDVFNAFRFTELGDVKVVILGQDPYHGPGQAHGLAFSVRPGIAIPPSLLNMYKELEN
H_sapiens         PPPHQVFTWTQMCDIKDVKVVILGQDPYHGPNQAHGLCFSVQRPVPPPPSLENIYKELST
                  ** .:**.  :   : ***************.*****.***:  :. **** *:****

H_influenzae      DISGFQMPSNGYLVKWAEQGVLLLNTVLTVERGMAHSHANLGWERFTDKVIAVLNEHREK
E_coli            TIPGFTRPNHGYLESWARQGVLLLNTVLTVRAGQAHSHASLGWETFTDKVISLINQHREG
H_sapiens         DIEDFVHPGHGDLSGWAKQGVLLLNAVLTVRAHQANSHKERGWEQFTDAVVSWLNQNSNG
                   * .*  *.:* *  **.*******:****.   *:** . *** *** *:: :*:: :

H_influenzae      LVFLLWGSHAQKKGQMIDRTRHLVLTAPHPSPLSAHRGFFGCRHFSKTNSYLESHGIKPI
E_coli            VVFLLWGSHAQKKGAIIDKQRHHVLKAPHPSPLSAHRGFFGCNHFVLANQWLEQHGETPI
H_sapiens         LVFLLWGSYAQKKGSAIDRKRHHVLQTAHPSPLSVYRGFFGCRHFSKTNELLQKSGKKPI
                  :*******:*****  **: ** ** :.******.:******.**  :*. *:. * .**

H_influenzae      DWQI-------
E_coli            DWMPVLPAESE
H_sapiens         DWKEL------
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